Effect of fresh leaf extract of tobacco on the somatic chromosome of *Allium cepa* Linn

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Abstract

The genotoxic potential of fresh leaf extract of tobacco was investigated by using chromosome aberration in *Allium cepa* root tip cells. *Allium cepa* roots were treated with 2g/lt., 3g/lt. and 4g/lt. concentrations of fresh leaf extract of tobacco and distilled water as control at 4 hours, 8 hours and 12 hours duration. The results indicated that fresh leaf extract of tobacco significantly increased the chromosomal abnormalities at all concentrations and treatment periods when compared with their controls and this increase was dose-dependent for the 4, 8 and 12 hours treatments. On the other hand, fresh leaf extract of tobacco significantly decreased the mitotic index (MI) in all treatments when compared with their controls. This study indicates that fresh leaf extract of tobacco decreased the mitotic index and produced clastogenic and aneugenic types of abnormalities in *Allium cepa* root tip cells. The data obtained in this study showed that chromosomal aberrations assay can be used as an important test battery to detect possible genotoxicity of chemicals in *Allium cepa*.

**Keywords:** *Allium cepa*, fresh leaf extract of tobacco, chromosomal aberrations, genotoxic effect, Mitotic index.

Introduction

It is well known fact that the structural and numerical changes of chromosome have been caused by a variety of physical and chemical agents which produce abnormalities both in the pattern of mitotic division and in the behavior of mitotic chromosome (Ohno, 1960; Ohno and Tanihuzi, 1960; Fiskesjo, 1969; Tarkowska, 1971; Sarker, 1974; Kabority et al., 1974; Amer and Farah, 1974, 1979; Badr, 1983; George and Geethamma, 1990; Saggoo et al., 1991; Karthikeyan, 1996; Anuratha, 2006; Arul raj, 2006). Pollution is a major problem which lowers the quality of life in various aspects. Environmental pollutions may be mutagenic or toxic for all living organisms (Yuzbasioğlu et al., 2008). Constant use of these chemicals may changing the hereditary constitution of an organism (Wuu and Grant, 1967; Wuu and Grant, 1966). When some chemicals accumulated within food chain to a toxic level, these chemicals affect directly the public health (Fisun and Rasgele, 2009). Treatment with Furadan and Endosulphan on *Allium cepa* root tip produced chromosomal aberrations such as strap nucleus, metaphase clumping, telomere puffing, scattered metaphase, multipolar anaphase, laggards, anaphase bridge, star anaphase, micronucleus, binucleate cell, C-metaphase, mature cell puffing, tropokinesis, nuclear lesion, nucleoids, cell death. These aberrations may lead to cancer, cell death mitotic delay and genetic changes (Manikandan, et al., 2013).
Materials and Methods

In the present investigation an attempt has been made to study the effect of Fresh Leaf Extract (Tobacco) on the somatic chromosome of Allium cepa Linn. Nicotiana tabacum Leaf Extract was used for the treatment of the root. The Tobacco Leaf was collected from the Tobacco merchant. The leaf contains the nicotine. It is very harmful to the plant materials. It causes the damages in the chromosome of Allium cepa L. The bulbs of Allium cepa L. were purchased from the vegetable market of Annamalai Nagar, Chidambaram, Tamil Nadu, India. The outer dried scales of bulbs were removed carefully with a blade. The bulbs were grown in clay pots filled with the garden soil, under identical environment conditions in the Botanical garden of Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. When the roots reached 1.5 - 2 cm in length, they were treated with different concentrations of tobacco leaf extract dissolved with distilled water (2 g/lt., 3 g/lt. and 4 g/lt.) for 4, 8 and 12 hours. Controls were also treated with distilled water for the same time periods. The concentrations were chosen according to their dose of application in agricultural field to control different diseases. For mitotic studies, the root tips of A. cepa were fixed in 1:3 acetic acid - ethyl alcohol mixture for overnight, followed by 5-7 minutes treatment in 45% acetic acid. Then root tips were hydrolyzed in 1 (N) HCL at 600C for 5 minutes, followed by staining with 2% aceto-orcein following the methods described by Sharma and Sharma (1980). After proper fixation and staining, appropriate squash preparations were made for each of the treatment and control. Effect of chemical treatment and control on different chromosome plates were observed under light microscope. To determine the effects of this chemical on mitotic index, 2000 cells were scored in control group and in each treated group. The mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells. Cytological abnormalities were also observed and scored. In this study a statistical analysis was done to estimate standard error (SE) of the results. Photomicrographs of cells showing chromosomal aberrations as well as showing normal mitosis were taken using Olympus microscope.

Results and Discussion

Microscopic examination of squashed Allium cepa L. root tip meristem cells showed that Tobacco fresh leaf treatments induced a number of mitotic abnormalities when compared with control. The increase of mitotic abnormalities was dependent on the increasing treatment periods and concentrations. The most common chromosomal abnormalities were stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-aphase, clumping and fragmentation. Tobacco fresh leaf caused a decrease in mitotic index (MI) at all the treatment groups. MI decreased in treated plants with different concentrations and treatment periods (Table-1).

Fresh leaf extract of tobacco is usually less toxic to plants when compared with chemical, industrial effluents, radiation etc. (Banarjee and Sharma, 1981). The toxic effects of fresh leaf extract of tobacco in plants include reduction in chlorophyll content. These are all due to presence of alkaloid-Nicotine’ which brings about C-mitosis distributed anaphase and chromosomal fragmentation. The alkaloid affect cells undergo C-mitosis exhibits chromosome which divide lengthwise and remain attached only at the region of contromere, as a result considerably shortening of chromosome. When two centric fragment unit which each other, a dicentric chromosome is formed. This dicentric chromosome is formed chromosomal bridges (Shanthamurthy and Rengaswamy, 1979). Mitotic index is an acceptable measure of cytotoxicity for all living organisms (Smaka-Kinel et al., 1996). The cytotoxicity level can be determined by the decreased rate of mitotic index. A decrease of mitotic index below 50% usually has lethal effects (Panda and Sahu, 1985). If mitotic index decreases below 22% of control, that it causes sub lethal effects on test organism (Antonsie-Wiez, 1990). According to many investigators, abnormalities due to inhibition of spindle formation such as c-mitosis, multipolarity, stickiness reflects high toxicity of pollutants (Lazareva et al., 2003; Kovalchuk et al., 1998; Hallem 1990; Amer and Ali, 1974). In the present study, Tobacco fresh leaf decreased the mitotic index at all concentrations and at all treatment periods when compared with control. The decrease of mitotic index was dose dependent. At all treatment periods, the highest concentration of Tobacco fresh leaf decreased mitotic activity more than other used concentrations. The percentage of mitotic index decreased with the increase of cells with c-mitosis, stickiness, laggards, anaphase and telophasic bridges etc. Since it decreased the MI in root tip cells of Allium cepa L. Tobacco fresh leaf can be accepted as a toxic agent in this study. Chromosomal stickiness is characterized by chromosomal clustering during any phase of the cell cycle. Stickiness and clumping may be caused by genetic and environmental factors. Several agents have been reported to cause chromosomal stickiness (Panneerselvam et al., 2012; Caetano-Pereira et al., 1998; Badr and Ibrahim, 1987).
In this study, exposed to blitox

<table>
<thead>
<tr>
<th>Times of treatment (hrs)</th>
<th>Conc.(g/L)</th>
<th>Mitotic Index (Mean±SE)</th>
<th>Mitotic abnormalities %</th>
<th>Total abnormality %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>L</td>
<td>C-M</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>18.15 ± 2.4</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>2</td>
<td>15.20 ± 2.2</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>3</td>
<td>11.31 ± 2.1</td>
<td>0.05</td>
<td>1.5</td>
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<td></td>
<td>4</td>
<td>9.90 ± 1.3</td>
<td>1.06</td>
<td>0.65</td>
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<tr>
<td>8</td>
<td>Control</td>
<td>15.35 ± 1.6</td>
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<td>2</td>
<td>10.90 ± 2.1</td>
<td>3.5</td>
<td>0.2</td>
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<td>8.71 ± 2.1</td>
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<td>12</td>
<td>Control</td>
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<td>4</td>
<td>6.21 ± 1.8</td>
<td>7.1</td>
<td>3.4</td>
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</tbody>
</table>

Abbreviations: S: Stickiness; L: Laggards; C-M: C-mitosis; B: Bridges; M: Multipolarity; P: Picnosis; S-A: Star-Anaphase; S-T: Star-Telophase; CL: Clumping

Gaulden (1987) postulated that sticky chromosomes result from the defective functioning of one or two types of specific non-histone proteins involving chromosome organization which are needed for chromatid separation and segregation. In this study, occurrence of several types of chromosomal abnormalities, such as stickiness, laggards, c-mitosis, bridges, multipolarity, picnosis, star-anaphase, star-telophase, clumping and fragmentation of *Allium cepa* L. root tip cells clearly shows that the accumulated effect of Tobacco fresh leaf results inactivation of spindle formation, deformation of non-histone chromosomal proteins and mutation of the structural genes. These results indicated that Tobacco fresh leaf should be regarded as a mutagenic agent for plants.

References


